36th Plant Development Workshop

SaturdayNovember 16, 2002

Organized by:
Vojislava Grbic
Tania Humphrey
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36th Plant Development Workshop Timetable

8:30 - 9:10 Registration, poster set up

9:10 - 9:20 Welcoming remarks

9:20 - 9:40 Larry Peterson
Root biology and microrizae lab

9:40 - 10:00 Ewa Cholewa, University of Waterloo
An unusual arrangement of xylem and phloem in the corm of *Eriophorum vaginatu*m allows for efficient nutrient relocation in adverse environments.

10:00 - 10:20 Krzystof Szczyglowski, Agriculture and Agri-Food Canada, SCPFRC, University of Western Ontario
Symbiotic development in legumes.

10:20 - 10:40 Manish Raizada, University of Guelph
Wound-Induced *de novo* Stem Cell (Meristem) Formation in *Arabidopsis*

10:40 - 11:20 COFFEE BREAK & POSTER GLANCING

11:20 - 11:40 Joe Colasanti, University of Guelph
Leaf-derived Floral Inductive Signals: Analysis of the Maize *id1* Gene

11:40 - 12:00 Dan Riggs, University of Toronto
KNAT1 and ERECTA regulate inflorescence architecture in Arabidopsis

12:00 - 12:20 VojislavaGrbic´, University of Western Ontario
Arabidopsis shoot patterning

12:30 - 2:00 LUNCH & POSTERS

2:00 - 2:20 Nancy Dengler, University of Toronto
Tissue pattern formation and leaf development.

2:20 - 2:40 Thomas Berleth, University of Toronto
Axis formation at the cellular, tissue and organismal level

2:40 - 3:00 Robin Cameron, University of Toronto
Age-related resistance: A novel, developmentally induced defense response that utilizes the signaling molecule, Salicylic Acid

3:00 - 3:20 Yukari Uetake, University of Guelph
Changes of cytoskeletal arrays in symbiotic orchid protocorms

3:20 - 3:50 COFFEE BREAK

3:50 - 4:10 Shengwu Ma, University of Western Ontario
Use of Transgenic Plants Expressing Autoantigen Glutamic Acid Decarboxylase (hGAD 65) and Regulatory Cytokine Interleukin-4 (IL-4) to Induce Oral Immune Tolerance to treat Autoimmune Diabetes

4:10 - 4:30 Fengshan Ma, University of Waterloo
Plant Roots and Seed Coats: Structure-Function Considerations

4:30 - 4:50 Barbara Moffatt, University of Waterloo
Accomodating the methylation requirements of plant development.

4:50 - ? Business Meeting

5:00 SOCIAL

Oral Presentation Abstracts

Ewa Cholewa and Marilyn Griffith
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An unusual arrangement of xylem and phloem in the corm of *Eriophorum vaginatum* allows for efficient nutrient relocation in adverse environments.

Eriophorum ssp. form the sedge tussock tundra and are the most abundant perennial graminoids in the Arctic. Ecological studies have shown that *Eriophorum* spp can dominate sites with infertile soils, and that they accumulated ^{137}Cs after the Chernobyl nuclear plant explosion and heavy metals solubilized from submerged mine tailings. All these studies indicate that the plants have an unusually high capacity to take up and retain ions. To better understand ion uptake and storage processes, we examined the anatomy of the overwintering storage organs of *E. vaginatum* and *E. scheuchzeri*. *E. scheuchzeri* produces a stolon that consists mainly of storage parenchyma cells which are protected by an epidermis and multiseriate exodermis. The collateral vascular bundles are vertically positioned in the middle of the stolon and the endodermis is absent. In contrast, *E. vaginatum* develops a ring of horizontally arranged xylem and phloem internal to the endodermis, in addition to vertical amphivasal vascular bundles leading to the leaves. As shown by the transport of fluorescein in the phloem and Safraine O in the xylem, each vertical bundle contacts the horizontal ring of vascular tissues so that solutes from one vascular bundle can move into the vascular ring and be circulated to another vascular bundle. In addition, special groups of cells with thick, lignified cell walls are associated with the basal terminus of each leaf trace within the corm. These cells aid both phloem (they retain fluorescein) and xylem (their cell walls stain with Safranine O) transport. This unique anatomy makes *E. vaginatum* a very distinctive "ecosystem engineer". The plant has developed a vascular system that is quite efficient in recycling of nutrients internally, which allows it to colonize sites with low fertility. Such adaptations may prove beneficial in adverse environmental conditions, as *E. vaginatum* is the only plant species to have survived crude oil spills in Alaska.

Krzysztof Szczyglowski[1,2], Bogumil Karas[2] and Lisa Amyot[1]
[1]Agriculture and Agri-Food Canada, SCPFRC, [2]University of Western Ontario, London, Ontario.

Symbiotic development in legumes.

With one notable exception, namely the genus *Parasponia* in the elm family, the ability to form nitrogen-fixing symbiosis with gram-negative soil bacteria known as rhizobia is restricted to the legume family, *Leguminosae*. While the biology of nitrogen-fixing root nodules has been broadly investigated, we still do not understand what unique evolutionary event predisposed legume plants and *Parasponia* to form nodular symbiosis with rhizobia. Interestingly, as more pieces are added to the nodulation puzzle, a common picture with non-symbiotic aspects of plant development emerges. It appears that nodules "have recruited" a number of pre-existing key plant regulatory programs and/or elements of these programs for their own development. As an example, the presentation will discuss a role of *L. japonicus* nodule autoregulation receptor kinase (NARK)[1] in long-range feed back regulatory mechanism(s) that establishes homeostasis of symbiotic and non-symbiotic root development in legumes.

1. Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski K, Duc G, Kaneko T, Tabata S, de Bruijn F, Pajuelo E, Sandal N, Stougaard J (2002) *Shoot control of root development and nodulation is mediated by a receptor-like kinase.* Nature advance online publication, 6 November 2002 (doi:10.1038/nature01207)

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Wound-Induced *de novo* Stem Cell (Meristem) Formation in *Arabidopsis*

Upon mechanical wounding, such as a herbivore attack, certain species of higher plants and many lower plants have a remarkable ability to regenerate new shoot and/or root stem cells (meristems) at the wound site. This can result in the formation of new shoots and/or roots. Our lab is trying to decipher developmental decisions during this process, to understand how the environment (e.g. light) enhances or inhibits new stem cell formation, and ultimately to isolate the key genes/alleles involved. By wounding 9000 cotyledons representing 60 ecotypes of *Arabidopsis thaliana*, we have examined the natural genetic variation for root and shoot regeneration in this species. We are using differences in regeneration between these ecotypes to map key loci and to understand the role of hormones and light in determining regeneration competency. To understand the stages of regeneration, we have begun utilizing developmental GUS/GFP markers, and we will use known mutants and transgenes which affect meristem formation, the cell cycle, hormone perception/signalling and light/carbon/nitrogen/wound perception and signalling.

Joe Colasanti,
University of Guelph

Leaf-derived Floral Inductive Signals: Analysis of the Maize *id1* Gene

Plants integrate both developmental and environmental signals to facilitate optimal growth and reproductive success. A clear example of this developmental flexibility is illustrated by the transition from vegetative to reproductive growth. At present almost nothing is known about the mechanisms that control the transition to flowering. Physiological studies have deduced that hormone-like substances produced in leaves mediate this important transition, however, the biochemical nature of putative signaling molecules remains a mystery. We are taking a molecular and genetic approach to understand the components of floral inductive signals by analysing the function of the maize *indeterminate* gene (*id1*). Mutant *id1* plants continue to grow vegetatively and are unable to undergo a normal transition to flowering. The *id1* gene encodes a putative transcription factor, suggesting that the *id1* gene product is an important regulator of the transition to flowering in maize. Expression analysis suggests that *id1* acts by controlling the production of a leaf- generated, transmissible signal that elicits the transition to flowering. Current research is focused on characterizing how the ID1 protein acts in leaves to regulate flowering.

C.D. Riggs, S.J. Douglas, G. Chuck, R.E. Dengler, and L. Pelecanda
Department of Botany, University of Toronto

KNAT1 and ERECTA regulate inflorescence architecture in Arabidopsis

Plant architecture is dictated by morphogenetic factors that specify the number and symmetry of lateral organs as well as their position relative to the primary axis. Mutants defective in patterning of leaves and floral organs have provided new insights on the signaling pathways involved, but there is comparatively little information on aspects of patterning of stems, which play a dominant role in architecture. To this end we have characterized five alleles of the brevipedicellus mutant of Arabidopsis, which exhibits reduced internode and pedicel lengths, bends at nodes, and downward-oriented flowers and siliques. Bends in stems correlate with a loss of chlorenchyma tissue at the node adjacent to lateral organs and in abaxial regions of pedicels. A stripe of achlorophyllous tissue extends basipetally from each node and is positioned over the vasculature that services the corresponding lateral organ. Map-based cloning and complementation studies revealed that a null mutation in the KNAT1 homeobox gene is responsible for these phenotypes, but only when present in an erecta background. Our observation that wild type Arabidopsis plants also downregulate chlorenchyma development adjacent to lateral organs leads us to propose that KNAT1 and ERECTA are required to restrict the action of an asymmetrically-localized, vasculature-associated chlorenchyma repressor at nodes. Our data indicates that it is feasible to alter the architecture of ornamental and crop plants by manipulating these genetically defined pathways.

V. Grbic, B. Poduska, T. Humphrey and A. Kalinina
Department of Biology, University of Western Ontario, London

***Arabidopsis* shoot patterning**

One of the main factors contributing to the plant morphological diversity is branching pattern. The complex branching pattern characteristic of the mature plant arises from developmental processes carried out by axillary meristems. Axillary meristem development can be described in terms of suppression, induction and identity. Axillary meristems form in the leaf axils in a predictable position at the leaf base. They can either develop concomitantly with the leaf, or their development can be initially suppressed, resulting in a full leaf development prior to the appearance of an axillary bud. Once established, the fate of axillary meristems is determined subsequently by an independent signaling cascade. The **long-term goal** of my research is to understand the molecular mechanisms that govern axillary meristem development by undertaking genetic, molecular and cell biological studies of axillary meristem development in a plant model organism Arabidopsis thaliana. The **short-term objective** of my research is to address following questions: (1) How are axillary meristems specified within the developing leaf? (2) What are determinants of the temporal pattern of axillary meristem formation? and, (3) What determines the fate of axillary meristems once they form?
The presentation will focus on studies of the naturally occurring variants of *Arabidopsis thaliana* that have altered shoot morphology, as they allow us to gain understanding on the developmental and genetic bases of the evolution of novel plant forms.

1. Poduska, B., Humphrey, T., Redweik A. and Grbic V. The *AERIAL ROSETTE 1* flowering gene underlies a novel morphology in *Arabidopsis. Genetics*, in press.
2. Kalinina, A., Mihajlovic, N. and Grbic, V. (2002) Axillary meristem development in the branchless *Zu-0* ecotype of *Arabidopsis thaliana. Planta* 215, 699-707.
3. Grbic, V. and Bleecker, A.B. (2000). Axillary meristem development in *Arabidopsis thaliana. Plant Journal* 21, 215-224.
4. Grbic, V. and Bleecker, A.B. (1996). A Unique Body Plan Is Conferred On *Arabidopsis* Plants Carrying Dominant Alleles Of Two Genes. *Development* 122: 2395-2403.

Dengler, Nancy*, Julie Kang, Riyadh Muhaidat, Athena McKown and Arunika Gunawardena.
Department of Botany, University of Toronto.

Tissue pattern formation and leaf development.

The purpose of this presentation is to provide an overview of current research in our lab. Our longest-term interest is the study of Kranz anatomy, a suite of structural specializations of photosynthetic tissues that invariably accompany C4 photosynthesis. Kranz anatomy is a striking example of convergence, since this syndrome has evolved at least 30 times during the diversification of the angiosperms. Riyadh Muhaidat is characterizing the structural, biochemical and physiological nature of this convergence in the genera *Haloxylon* and *Calligonum*, both woody plants from the deserts of central Asia that have similar photosynthetic tissue arrangement, but belong to unrelated families. Alteration of vein spacing is the most consistent feature of Kranz anatomy across all lineages where it has evolved. Athena McKown is examining how the development of leaf vein pattern is altered in species of *Flaveria*, a genus of the Asteraceae with C3, C4, and intermediate species. Julie Kang studies the role of cell cycling during leaf vein pattern development in *Arabidopsis*. She has shown that leaf vein procambium has a unique pattern of cell cycling that distinguishes vein precursors from adjacent tissues and results in the hierarchical pattern of venation. The pattern of cycling coincides with the pattern of expression of one of the earliest markers of procambial identity, the HD-ZIP homeobox gene *ATHB-8*. Arunika Gunawardena studies the role of programmed cell death in tissue pattern formation and in leaf morphogenesis, including the genera *Monstera* and *Aponogeton* that achieve a complex leaf shape through the patterned death of specific tissue regions.

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Axis formation at the cellular, tissue and organismal level

In most organisms, body axes are established at an early embryo stage and provide a framework for subsequent patterning processes. In plants, genetic and experimental evidence suggests common mechanisms underlying the initiation of the embryonic axis, the continuous organization of vascular tissues and the acquisition of cell polarity in general. A number of resent results suggest apical-basal auxin transport as a basic orienting signal and define more precisely a developmental role of auxin in local patterning events.
Mutations in the *Arabidopsis* gene *MP* strongly interfere with auxin perception, embryo axis formation and vascular tissue continuity. The *MP* gene encodes an "Auxin Response" transcription factor capable of recognizing functional control elements in auxin inducible promoters. Microarray data identify genes with *MP* dependent auxin inducibility, some of which are involved in auxin transport and early vascular differentiation. Double mutant and protein interaction studies identify redundant and non-redundant interaction of MP and the related transcription factor NPH4.
Auxin response reporter gene expression patterns indicate auxin accumulation in provascular tissue prior to overt differentiation. Experimentally induced shifts in the position of these local auxin maxima result in correspondingly altered venation patterns, indicating an instrumental role of auxin in vascular differentiation in organogenesis

K. Zaton, **R.K. Cameron**
Department of Botany, University of Toronto

Age-related resistance:
A novel, developmentally induced defense response that utilizes the signaling molecule, Salicylic Acid

Age-related resistance (ARR) is a form of resistance that develops in mature *A. thaliana* plants in response to *Pseudomonas syringae* pv. *tomato* (*Pst*) infection. The salicylic acid (SA)-accumulation deficient lines NahG, *sid1*, *sid2*, and *pad4* demonstrate the importance of SA accumulation during ARR by their inability to display this defense response. Previous studies demonstrate that PR-1 gene expression does not correlate with ARR and that ARR is a defense response distinct from induced systemic resistance or systemic acquired resistance in that it occurs in a number of defense response mutants (*npr1*, *pad3-1*, *eds7-1*, *etr1*) (Kus *et al.*, Plant Cell 14, 2002, pg 479-490). *Pst* reside in the intercellular spaces of infected plants and intercellular washing fluids (IWFs) collected from plants expressing ARR exhibit *in vitro* anti-bacterial activity to *Pst*.
SA may act as a signal for ARR-associated defense or it may itself possess anti-microbial activity. We are currently in the process of analyzing intercellular SA levels during ARR using HPLC. Recent experiments in which the SA-degrading enzyme salicylate hydroxylase was injected into leaves following bacterial inoculation suggest that SA may have direct anti-bacterial effects.

Yukari Uetake and R. Larry Peterson:
University of Guelph

Changes of cytoskeletal arrays in symbiotic orchid protocorms

Mycorrhizal fungal associations with orchids are essential in seed germination and protocorm development. Fungal hyphae invade embryo cells and form hyphal coils called pelotons; subsequently these senesce and collapse. Intact and collapsed hyphae are surrounded by host plasma membrane. Microtubules and actin filaments are components of the cytoskeleton of plant cells. Involvement in cell morphogenesis and organelle movement and the alterable nature of this system by external influences, have aroused interest in the roles of the cytoskeleton in plant-microbe interactions. In this study, changes in microtubule and actin filament arrays accompanying fungal colonization and senescence were studied in *Spiranthes sinensis* protocorms symbiotically cultured with *Ceratobasidium cornigerum*. Techniques used were immunofluorescence method combined with confocal microscopy, conventional TEM and immunogold labelling. Both microtubule and actin filament arrays in the cortical cells of the symbiotic orchid protocorms are altered accompanying fungal colonization and senescence. The close spatial relationship between microtubules and the perifungal membrane, and between actin filaments, ER and the perifungal membrane in this study suggest important roles of these structures in the functioning of the interface between the fungus and host cytoplasm in this symbiosis.

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Use of Transgenic Plants Expressing Autoantigen Glutamic Acid Decarboxylase (hGAD 65) and Regulatory Cytokine Interleukin-4 (IL-4) to Induce Oral Immune Tolerance to treat Autoimmune Diabetes

The induction of immunological unresponsiveness by feeding soluble antigens, termed oral tolerance, has been attracting considerable attention as a potential therapy for a variety of systemic inflammatory disorders such as autoimmune diabetes. However, as oral tolerance induction requires the repeated administration of large quantities of protein antigens, the clinical success of this approach as a therapy may be critically dependent on: 1) the availability of relevant protein antigens in large quantities and at low cost, and 2) the establishment of an effective and simple delivery system. Previously we demonstrated that transgenic plants could be an exciting approach of combining an economic production method with an oral delivery system for a diabetes-associated autoantigen, the mouse glutamic acid decarboxylase (GAD67), to induce oral immune tolerance to prevent autoimmune diabetes in the non-obese diabetic (NOD) mouse, which serves as a model for human diabetes. Use of adjuvants may enhance oral immune tolerance, but commonly used mucosal adjuvants such as cholera toxin B subunit (CTB) may be limited by neutralizing immune responses. Immunoregulatory cytokines such as interleukin-4 (IL-4), which promotes Th2 immune response but unlike CTB, is of natural-occurring self-immune component, may also exhibit mucosal adjuvant activity for the induction of oral tolerance to co-administered tolerogenic antigen. In this presentation, the expression of human GAD65 and murine IL-4 in plants will be described. The effect of plant-derived IL-4 on the enhancement of oral tolerance will be discussed including its synergy with plant-derived GAD65 in preventing diabetes in NOD mice. Transgenic plants expressing an autoantigen and an immunoregulatory Th2 cytokine may represent a new therapeutic strategy for treating autoimmune diseases in humans.

Peterson, CA and **Ma FM**
Department of Biology, University of Waterloo, Waterloo, ON

Plant Roots and Seed Coats: Structure-Function Considerations

Transport processes constitute the general theme of research in our laboratory. For this, we correlate structure and function, often with the help of fluorescence microscopy. The exodermis of roots is a major interest, and we continue to investigate its structure, function, development, control of development, and its interactions with endomycorrhizae. Maize roots provide a model system for the uniform exodermis, and onion roots for the dimorphic exodermis, and we have studied roots of many other species to a lesser degree. With this basic knowledge of root structure, we can understand roots' reactions to stress, and also predict pathways of movement from the soil solution to the xylem for water and ions into roots. The pathway of calcium is still under debate. By using a variety of techniques (localized feeding, compartmental elution, inhibitor studies, modeling membrane surface areas and Ca^{2+}ATPases) we determined that this ion crosses the endodermis and exodermis through the symplast. Tree roots have not escaped our scrutiny, and we have divided individual roots into three areas that should have varying capacities for water and ion uptake. Recently, we have embarked on a study of soybean seed coat anatomy and permeability as part of a larger, collaborative project.

Barbara Moffatt,
University of Waterloo

Accomodating the methylation requirements of plant development.

My students and I are investigating how the methylation activities of plant cells, such as those involved in the synthesis of lignin, pectin and phosphocholine, or the modification of nucleic acids or proteins, are maintained throughout plant development. There are hundreds of different methyltransferases and virtually all of these rely on S-adenosylmethionine as the methyl donor. Plant tissues differ in their methylation requirements throughout development and we seek to learn how these demands are monitored and satisfied. We are currently focusing on two specific enzyme actitivities, adenosine kinase (ADK) and S-adenosylhomocysteine hydrolase (SAHH), which are involved in recycling the by-products of SAM-dependent methylation reactions and which appear to change in association with changing methylation needs. We are investigating where these enzymes are localized, the metabolic signals affecting their expression and transcriptional changes in ADK and SAHH mutants. A brief overview of our progress and future plans will be presented along with our working model of how the pathway may be regulated.

Poster Abstracts

Vojislava Grbic, Anna Kalinina, Nela Mihajlovic and Esther Hidber
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Axillary meristem development in the branchless Zu-0 ecotype of *Arabidopsis thaliana*.

Axillary meristems form in the leaf axils during post-embryonic development. In order to initiate the genetic dissection of axillary meristem development, we have characterized the late-flowering branchless ecotype of *Arabidopsis*, Zu-0. The oldest rosette leaves of Zu-0 plants all initiate axillary meristems, but leaves from the upper part of the rosette remain branchless. Alteration in the meristem development is axillary meristem specific because the shoot apical and floral meristems develop normally. Scanning electron microscopy, histology and RNA in situ hybridization with *SHOOTMERISTEMLESS* (*STM*), a marker for meristematic tissues, show that a mound of cells form and *STM* mRNA accumulates in barren leaf axils, indicating that axillary meristems initiate but arrest in their development prior to organizing a meristem proper. A genetic analysis suggests that the branchless phenotype arises due to a single recessive allele whose effect on the branching pattern of Zu-0 plants can be suppressed by early flowering.

Yosr Z. Haffani, Nancy F. Silva & Daphne R. Goring

PERK1, A NOVEL RECEPTOR-LIKE PROTEIN KINASE, ENHANCES THE OVERALL DEVELOPMENT IN THE TRANSGENIC *Arabidopsis thaliana* L. PLANTS

Plants are subjected to a variety of external stimuli and have evolved mechanisms responsive to environmental changes. Receptor-like protein kinases (RLKs) play a fundamental role in the perception and transmission of a stimulus through a signalling cascade to elicit appropriate cellular responses. PERK1 (Proline Extensin-like Receptor Kinase 1) encodes a novel RLK in *Brassica napus* and constitutes a novel class of plant receptor-like protein kinases. The PERK1 protein consists of a proline-rich extracellular domain showing sequence similarity to extensins, a unique transmembrane domain and a catalytic domain with serine/threonine kinase activity. It was demonstrated that PERK1 is strictly localized to the plasma membrane of onion epidermal cells by biolistic bombardment using a PERK1-GFP fusion protein. The role of PERK1 in mediating plant defense responses has been investigated and showed that PERK1 mRNA levels dramatically and rapidly accumulated after various wounding stimuli and increased moderately in response to fungal pathogen treatment.

More recently, functional analysis studies in transgenic *Arabidopsis thaliana* (Col-0) suggested that PERK1 may also have a role in plant development. The overexpression of the PERK1 cDNA under the control of the strong 35S cauliflower mosaic virus (CaMV) promoter showed several heritable traits including enhanced growth and fertility. Three independent lines show increased height, secondary branching and seed production compared to the wild-type *Arabidopsis* plants. Identifying upstream regulators and downstream targets is becoming the newest challenge in PERK signalling research.

Hayter ML and Peterson CA.
Department of Biology, University of Waterloo, Waterloo, ON.

Feasibility of symplastic Ca^{2+} movement through the root endodermis and exodermis

How do Ca^{2+} ions pass through the endodermis and exodermis of roots? If a symplastic route is used, the limiting step would be unloading from the protoplasts with Ca^{2+}-ATPases. In the endodermis this unloading occurs on the stele side of the Casparian band, and in the exodermis it occurs on the cortical side of the Casparian band.
According to Philip White, the membrane surface areas involved are too small to hold enough protein to transport sufficient Ca^{2+} to the plant. To test this assertion for onion (with known delivery rates of the ion to the stele), measurements were made of the relevant surface areas. Protein densities in an endodermal plasma membrane and a range of Ca^{2+}-ATPase activities were taken from the literature. A number of models were constructed to determine what percentage of the total membrane protein would need to be Ca^{2+}-ATPase to account for the observed Ca^{2+} fluxes. For the endodermis, this percentage is 14-68%; adding the surface area of the pericycle reduces this range to 7-34%. For the exodermis, the range of the percentage is 23-115%; adding the surface area of the adjacent cortical cells reduces it to 1-6%. It is concluded that the symplastic transport of Ca^{2+} through both the endodermis and exodermis cannot be discounted on the grounds of insufficient plasma membrane protein.

Anna Kalinina, Nela Mihajlovic and Vojislava Grbic
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The role of *LEAFY* in determination of the primordia initiation rate and activation of axillary meristems

At the transition to reproductive development several processes occur concomitantly. Firstly, the primary shoot apical meristem ceases initiation of leaf primordia and starts initiation of floral meristems; secondly, axillary meristems initiate in the axils of the youngest leaf primordia in a basipetal pattern, and thirdly, the rate of organ initiation increases. Simultaneous occurrence of these processes in *Arabidopsis* raises the possibility that they are coordinately regulated. The transcriptional factor *LFY* promotes floral meristem development by activating floral meristem identity genes. This study uses *lfy1*, a loss-of-function allele, and gain-of-function the 35S::*LFY* construct to investigate whether *LFY* also plays a role in establishment of the elevated primordia initiation rate and activation of axillary meristems. Our results indicate that LFY is required for maintenance of the higher rate of primordia initiation and that *LFY*, together with the independently acting factor, can activate axillary meristem development.

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Two Types of Mineral Nutrient Storage Particles in Embryos of Seeds from Phyla Coniferophyta, Cycadophyta, Gnetophyta and Ginkgophyta: Early Seed Plant Characteristic.

Mature seeds of flowering plants contain mineral nutrient storage particles called globoids that are rich in phosphorus, potassium and magnesium. Globoids, which occur inside protein bodies, may also contain some amounts of calcium, iron, manganese and zinc (usually as trace amounts). Published research from this laboratory demonstrated that seeds from different genera in the conifer Family Pinaceae contained two different mineral nutrient rich particles, namely globoids and iron-rich particles. The iron-rich particles are rich in iron, phosphorous, and potassium. These particles occur inside plastids may also contain some magnesium and perhaps chlorine. We used energy dispersive x-ray analysis and transmission electron microscopy to study the structure and element content of particles in embryo axis tissue from seeds of other gymnosperms. Both globoids and iron-rich particles were located in representatives of phyla Cycadophyta, Ginkgophyta, Gnetophyta and Coniferophyta (Families Araucariaceae, Cephalotaxaceae, Cupressaceae, Podocarpaceae, Sciadopityaceae and Taxaceae). Two types of mineral nutrient rich structures in seeds seems to be the ancestral condition for seed producing plants. Iron-rich particles appear to have been lost from seeds during evolution of the Anthophyta.

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Analysis of Limburg; an Arabidopsis late flowering aerial rosette bearing ecotype

Flowering time is regulated by a complex genetic network that integrates environmental cues with the developmental state of the plant. To elucidate the molecular mechanism of flowering in Arabidopsis we investigate the genetic bases underlying the formation of aerial rosettes in late-flowering Arabidopsis ecotypes such as Limburg (*Li*). Delayed transition to reproductive development in *Li* plants can be suppressed by vernalization, and the transition to reproductive development can be completely abolished if plants are grown under a short photoperiod. These physiological responses suggest that *Li* carries genes that act through the autonomous flowering pathway. Genetic analyses indicates that dominant alleles of two loci are responsible for the late -flowering aerial- rosette bearing phenotype. These loci have been identified as new alleles of the late flowering genes *FRI* and *FLC*. There is evidence that an additional genetic factor aerial rosette3 (*ART3*) may be required for the extreme late flowering phenotype of *Li* plants. This evidence includes: the extended linkage between the late flowering aerial-rosette phenotype to the distal 60cM of chromosome V, and variable flowering behavior of the *fri-Ler fri-Ler FLC-Li FLC-Li* lines (they either flower early, producing ~ 15 leaves, as typical for other *FLC*-containing lines, or after producing ≥ 40 leaves). Results of the experiments aimed toward identification and characterization of the *ART3* locus will be presented.

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Systemic acquired resistance and Age-related resistance, two distinct induced resistance pathways that both require salicylic acid.

Age-related resistance (ARR) is observed in mature *Arabidopsis* in response to *Pseudomonas syringae* pv. *tomato* (*Pst*) infection. Unlike ARR, systemic acquired resistance (SAR) is elicited in response to certain necrotizing infections in one part of a plant resulting in resistance to subsequent virulent infections in distant parts of the plant. Salicylic acid (SA) accumulation is necessary in many plant defense responses including SAR, but its mechanism of action is still unknown. Studies in our lab using plant lines which do not accumulate SA (*NahG, sid1, sid2*) demonstrate that SA accumulation is also important for ARR and intercellular washing fluids (IWFs) from plants expressing ARR exhibit anti-bacterial activity to *Pst*, suggesting that SA may accumulate in intercelluar spaces and act as an anti-microbial agent. The SAR-defective mutant *dir1-1* can perceive the SAR signal present in petiole exudates (enriched for phloem sap) from wild type SAR-induced plants, but *dir1-1* exudates do not contain this signal. Western analysis demonstrated that DIR1 protein is present in petiole exudates of SAR-induced wild type, but not *dir1-1* plants, suggesting that *DIR1* (putative lipid transfer protein) may be involved in the production of the SAR mobile signal or in transporting a lipid signal to distant tissues to establish SAR. Ongoing SAR and ARR experiments will be discussed.

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***Arabidopsis* Proline Extensin-Like Receptor Kinase (*At*PERK) Family And Their Function In Plant Defense Responses**

Plant receptor kinases form one of the most abundant protein classes that are able to sense changes at the cell surface and initiate appropriate responses through signal cascades. The PERK family in *Arabidopsis thaliana* consists of 14 genes identified by amino acid sequence similarity to the *Brassica napus* PERK1, a novel receptor kinase that appears to play a role in the wound response and in plant development (*Silva & Goring, Plant Mol Biol*, 2002). RNA gel blot analysis shows that the PERK family members are differentially expressed during development. The mRNA levels of *At*PERK1 (the PERK1 orthologue in *A. thaliana*) increase dramatically upon wounding as does that of *BnPERK1*. *At*PERK1 is also up-regulated within 10 hours upon inoculation with *avirulent Pseudomonas syringae* pv *tomato* (*Pst*). This suggests that *At*PERK1 may be involved in the early signal perception or transduction pathway of the wounding response and R-gene mediated resistance. Future experiments to investigate the function of *At*PERK family during plant defense responses will be discussed.